Effects of Poly I-C on the Course of Infection with *Trypanosoma cruzi* (34904)

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(Introduced by S. Baron)
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The antiviral activity of interferon is well documented for RNA and DNA viruses (1). Protection by means of interferon has also been obtained against malarial infections (2). Induction of interferon by *Toxoplasma gondii,* an intracellular protozoon, has been reported (3, 4), as well as a protective effect against this infection in chick and mouse cell monolayers (5). With other intracellular protozoa such as *Leishmania donovani,* attempts to protect against this infection with interferon inducers both in animals and in tissue culture, have failed (6). An interferon-like inhibitor was found in the serum of mice infected with *Trypanosoma cruzi,* the protozoan agent of Chagas' disease (7).

This paper reports the effects of a synthetic polynucleotide, the double-stranded polyinosinic–polycytidilic acid (Poly I-C), upon the infection of mice and tissue culture monolayers with *T. cruzi.*

Materials and Methods: Strain of T. cruzi. The Tulahuen strain of *T. cruzi,* kindly provided by Dr. I. G. Kagan, National Communicable Disease Center, was used. Suspensions of the parasite were made either from culture in liver infusion tryptose medium (LIT) (8), from the supernatant medium of infected tissue cultures, or from the blood of infected mice.

Mouse inoculation. Four-week-old female white mice of the Bagg Swiss strain raised at the Puerto Rico Nuclear Center Colony, with an average weight of 18 g, were used. The mice treated with Poly I-C received three intraperitoneal (ip) doses of 100 μg each at 12-hr intervals previous to the *T. cruzi* inoculum. Thereafter, they were injected with 100 μg of Poly I-C, 3 times a week during 3 weeks. The control group received parallel injections of the phosphate buffered saline (PBS) diluent. The *T. cruzi* inocula ranged from 10^5 thru 10^4, 10^3, and 10^2 blood forms which were injected ip to groups of 10 mice respectively.

Tissue culture system. The cell line DC2 was used (9). The cells were trypsinized, seeded in 13 × 130-mm screw-capped plastic tubes, and incubated at 37° with basal medium Eagles (BME) containing 10% calf serum plus 50 units of penicillin and 50 μg of streptomycin/ml. When the cells reached confluency (between 3 and 5 days) the medium was replaced by BME containing only 5% calf serum and antibiotics. The tubes were inoculated each with 0.1 ml of the Poly I-C solution (100 μg) and further incubated at 37° during 24 hr. They were inoculated with 0.1 ml of tenfold dilutions of a *T. cruzi* culture suspension containing 5 × 10^6/ml and reincubated at 37°. The medium was changed 3 times a week, and new Poly I-C was added with each change. This schedule was then continued for 3 weeks. The tubes were observed daily with an inverted microscope until the trypanosomal forms were seen in the culture fluid.

Determination of parasite levels. 1. Direct count of blood parasites. In order to obtain comparable parasitemia levels, the treated and control groups of mice were tail-bled at the same postinoculation time. The tail was cleaned with alcohol, thoroughly dried and its tip was cut. A small drop of blood was gently squeezed out and a 1:100 dilution was made by sucking blood and PBS diluent into hematocytometer diluting pipettes. Counting chambers were filled with the sus-

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pensions and the motile parasites in a 1.0 mm^3 volume were counted.

2. *Titration of parasites in spleen* (10). The spleen of moribund mice were taken aseptically, weighed, cut, and washed with Hanks' saline solution. The organs of 5 mice were pooled together, ground in a tissue grinder and tenfold dilutions (w/v) were made in EBM. From each dilution 0.1 ml was inoculated in each of 4 roller tubes with DC2 monolayers. The tubes were observed daily with the inverted microscope in search of extracellular trypanosomes. The tissue culture infective dose 50% ($TCID_{50}$) was computed by the Reed and Muench method (11).

Poly I-C. The double-stranded Poly I-C was prepared by combining equimolar quantities of both acids and dissolving them in a buffered saline solution in order to obtain a concentration of 1000 μg/ml. The preparation was frozen and kept at —20° until the moment of use.

Results. A. In vivo *experiments.* The effect of Poly I-C against *T. cruzi* infection in mice was measured in terms of death, parasitemia, and parasite load.

1. *Death rate.* Table I shows the results of a typical experiment in which tenfold dilutions of infected mouse blood were inoculated. Except in the control group inoculated with the lowest number of parasites (10^2) where 2 of the 10 mice survived into a chronic form of the disease, all the mice died with the infection. The death rate for the 10^5, 10^4, and 10^3 inocula were identical, but the mean survival time was always shorter in the treated groups than in the control ones, and this difference is significant ($p < 0.01$).

TABLE II. Parasitemia Levels in Poly I-C Treated and Control Mice Infected with *T. cruzi*.

Parasites/inoculum	Poly I-C	PBS	*p* Values
1×10^4 [a]	2.1[b]	3.4	>0.5
1×10^3	1.4	0.5	<0.01
1×10^2	9.9	0.95	<0.01

[a] Parasitemia in groups 1×10^4 and 1×10^3 was determined 12 days after inoculation. In group 1×10^2 it was done on the 17th day postinoculation.

[b] Million parasites per ml of blood.

2. *Parasitemia levels.* The presence of parasites in the blood of animals infected with *T. cruzi* is due to the rupture of the invaded cells and represents a rough indicator of the course of the infection. Table II shows the parasite counts done at the same time on the control and treated groups. The counts on the 10^4 and 10^3 groups were done on the 12th day postinoculation when the mice in these two groups were at the terminal stage of infection; the values found reflect significant differences only in group 10^3 ($p < 0.01$). Counts on the 10^2 groups were done on the 17th day postinoculation which was only 1 to 2 days before all the mice in the treated group died while the control animals were 10 days away from the average death time. As shown in Table II, the parasite

TABLE I. Effect of Poly I-C on Infection, Death Rate, and Survival Time of Mice Inoculated with *T. cruzi*.

	Poly I-C[b]			PBS			
Parasites/ inoculum[a]	Infectivity[c]	Death	Survival (days)	Infectivity	Death	Survival (days)	*p* Values for survival
1×10^5	10/10	10/10	12.0	9/9	9/9	14.6	<0.01
1×10^4	9/9	9/9	13.6	10/10	10/10	15.4	<0.01
1×10^3	10/10	10/10	14.8	9/9	9/9	18.1	<0.01
1×10^2	10/10	10/10	18.5	10/10	8/10	20 to chronic	<0.01
0	—	0/10	—	—	0/10	—	

[a] Blood parasites diluted in Hanks' solution and inoculated by the ip route in 0.25-ml amounts.

[b] Three doses ip of 100 μg of Poly I-C each at 12-hr intervals previous to *T. cruzi* inoculum, with 100 μg 3 times a week during 3 weeks.

[c] Mice infected/mice inoculated.

TABLE III. Intracellular Multiplication of *T. cruzi* in DC2 Cell Monolayers Treated with Poly I-C.

Treatment of cells	Trypanosomes/inoculum[a]					
	5×10^4	5×10^3	5×10^2	5×10^1	5×10^0	5×10^{-1}
Poly I-C (2)	4/4[c]	4/4	4/4	4/4	4/4	0/4
Control	4/4	4/4	4/4	4/4	4/4	1/4

[a] Parasites from cell monolayer, 5×10^6/ml. Four tissue culture tubes were inoculated each with 0.1 ml.

[b] One hundred μg of PIC was administered to the cells 24 hr before inoculation. The same amount was given 3 times a week as the medium was changed.

[c] Tubes showing parasites/tubes inoculated.

count in the treated group was 9.9×10^6 and 9.5×10^5 in the control mice ($p < 0.01$).

3. *Load of parasites in spleen.* The results of the tritations in DC2 monolayers of spleen pools of the Poly I-C-treated mice showed an infective titer of $10^{7.5}$ $TCID_{50}$/g of spleen while the control group was $10^{6.7}$. This difference is significant ($p < 0.01$).

B. In vitro *experiments in tissue culture.* The results shown in Table III indicate that intracellular multiplication of *T. cruzi* in DC2 monolayers is not affected by the relatively high levels of interferon induced in these cells in response to treatement with Poly I-C (6).

C. In vitro *experiments in LIT.* Poly I-C exhibits toxicity both for animals and cells. Therefore, an experiment was carried out to determine whether this property could affect the multiplication of *T. cruzi* in vitro. As shown in Table IV, Poly I-C in concentration up to 500 μg/ml of culture medium does not have any inhibitory effect on the growth of this protozoon. Moreover, the infectivity of the microorganisms obtained from these cultures was not affected as is demonstrated by titrations done in tissue culture monolayers.

Discussion. The results obtained as well as the similar ones reported by Worthington *et al.* (12), indicate that interferon induced by double-stranded Poly I-C does not have a protective effect on *T. cruzi* infections. Poly I-C, on the contrary, has a negative effect that might be the result of its toxicity. This is evidenced by the earlier appearance of parasites in the blood, shorter survival time, and the higher infectivity titers of the spleens of the treated mice. The greater levels of parasites reported in the infection of the intracellular protozoan *L. donovani* (6) could equally be explained by the toxic effects of Poly I-C. This negative effect, however, could not be detected in tissue cultures, suggesting that Poly I-C has a broader effect in the animal.

The lack of protection of interferon against *T. cruzi* infections can be explained by the difference between the metabolism of this parasite and the viruses. Interferon probably acts on the synthesis of viral proteins by interfering with the translation of viral messenger RNA from the cell ribosomes, but without affecting the cellular messenger RNA function (13–15). In the case of *T. cruzi* as well as certain other intracellular protozoan parasites, protein synthesis is regulated by

TABLE IV. Effect of Poly I-C on *T. cruzi* Growth *in Vitro* and Infectivity for Cells.

Effect observed on	LIT medium + Poly I-C (μg/ml)				
	0	1	10	100	500
Growth	+	+	+	+	+
Infectivity titer in DC2 cells/0.1 ml of growth medium	10^4	10^4	10^4	10^4	10^5

the parasite ribosomes, not by the cell host. If this is the case, interferon would not have any effect on the parasite protein synthesis, as is suggested by the results of our experiments as well as those of other authors.

The antiparasitic action of interferon inducers and interferon on *Plasmodium berghei* and *T. gondii* infections must be explained by other mechanism. Since both parasites possess their own ribosomes, the antiparasitic action, if it is indeed due to interferon, could be explained by the action of the "antiviral" protein, as suggested by Remington and Merigan (5), or by the dependence of the parasites on the cell ribosomes at certain states in which the parasite ribosomes are not fully developed. Whatever the mechanism of action, the antiparasitic effect provides a system in which, as pointed out by Jahiel *et al.* (2), the relative roles played by the ribosomes of the cell and the parasite in the action of interferon could be determined.

Summary. Results are presented on the effect of double-stranded Poly I-C upon infections with the intracellular parasite *T. cruzi.* The ip administration of 300 μg of Poly I-C previous to the inoculation of 1.25×10^3, 1.25×10^2, 1.25×10^1, and 1.25 mouse LD_{50} of the parasite, followed by doses of 100 μg, 3 times a week for 3 weeks, did not affect the lethality of the infection. On the contrary, the animals in the untreated groups survived by several days those of the treated groups and parasitemia and tissue invasion was of a lower order in the untreated groups. Similar negative results were observed in tissue cultures treated with 100 μg/ml of Poly I-C and infected with 10^3 $TCID_{50}$ of *T. cruzi.* It is interesting to note that doses of Poly I-C as high as 500 μg/ml did not affect the growth rate of *T. cruzi* in the *in vitro* culture medium, and that its infectivity was unaltered.

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